Original Article

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Comparison of the antimicrobial spectrum and mechanisms of organic virgin coconut oil and lauric acid against bacteria

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Abstract

Organic virgin coconut oil (VCO) contains almost 50% lauric acid (LA) . As lauric acid exhibits antimicrobial activity against some bacteria, VCO is thought to also possess antibacterial properties. However, it is unclear whether the antimicrobial activity of VCO is comparable to that of LA. The present study was performed to examine whether VCO demonstrates antimicrobial activity against species of gram-positive bacteria (i.e., Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus sanguinis, Streptococcus salivarius, and Streptococcus mutans) as well as LA by disk diffusion antibacterial test. Although LA has antimicrobial activity against S. aureus, S. pyogenes, S. agalactiae, S. mutans, and S. sanguinis, VCO has antimicrobial activity against S. pyogenes, S. agalactiae, S. mutans, and S. sanguinis, but not S. aureus. Furthermore, the antimicrobial activities of VCO against several Streptococcus species were weaker than those of LA. We further compared the antimicrobial activities of VCO and LA against Streptococcus pyogenes by antimicrobial test involving the inhibition of microbial growth in broth medium. While > 4.4 mM VCO was capable of exhibiting an antimicrobial effect against S. pyogenes, the same effect was demonstrated by as little as 0.18 mM LA. Furthermore, > 0.88 mM LA, but not VCO, was able to eliminate S. pyogenes completely. We also confirmed that LA could eliminate bacteria within 10 minutes, and the number of bacteria did not increase for 2 hours. On the other hand, the addition of VCO did not decrease the number of bacteria. In addition, scanning electron microscopic (SEM) analysis indicated that the antimicrobial activity of LA is mediated by a bactericidal mechanism, whereas VCO functions by inducing bacteriostasis. Taken together, we found that VCO has antimicrobial properties against some strains of bacteria belonging to the genus Streptococcus, but not Staphylococcus aureus or some gram-negative bacteria. These findings suggest that the antimicrobial spectrum of VCO differs from that of LA. We also found that the antimicrobial effect of VCO is mediated by bacteriostasis, and not a bactericidal mechanism as observed for LA.

KEY WORDS

Organic virgin coconut oil (VCO) , Lauric acid (LA) , Streptococcus pyogenes, Antimicrobial activity, Antimicrobial mechanism

Introduction

Recently, there have been several reports describing the health benefits of organic virgin coconut oil (VCO) [1・2・3・4] . The majority of the saturated fats found in VCO are medium-chain fatty acids, which do not undergo degradation and re-esterification processes and are directly used in the body to produce energy [5] . Coconut oil, including VCO, decreases cardiovascular risk factors and provides enhanced protection against heart disease [2] . VCO may be an efficient nutraceutical

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in preventing the development of diet-induced insulin resistance and its associated complications, probably because of the antioxidant properties of VCO [6]. Overall, the literature suggests that consuming VCO promotes good health.

VCO contains eight medium-chain fatty acids: 1) lauric acid (LA); 2) myristic acid; 3) palmitic acid; 4) caprylic acid; 5) capric acid; 6) stearic acid; 7) oleic acid; and 8) linoleic acid, some of which have demonstrated antibacterial effects [7]. LA has exhibited potent antibacterial activity against some Gram-positive bacterial species (i.e., Staphylococcus, Streptococcus and Clostridium) [7·8·9·10]. Furthermore, several reports have demonstrated that caprylic acid, capric acid, myristic acid, and oleic acid have antibacterial properties against bacteria and fungi [7]. As VCO contains almost 50% LA and a substantial amount of antibacterial fatty acids, it is also thought to exhibit antibacterial activity. However, there is little evidence of the antibacterial properties of VCO against Gram-positive strains of bacteria. In addition, some reports have shown that VCO displays low antibacterial activity against Staphylococcus aureus [11·12]. Therefore, it remains to be determined whether VCO possesses antibacterial properties similar to LA.

In the present study, we determined whether VCO demonstrates antimicrobial activity against species of Gram-positive bacteria (i.e., Staphylococcus aureus, Streptococcus pyogenes, S. agalactiae, S. sanguinis, S. salivarius, and S. mutans). Furthermore, we performed a detailed analysis of the antibacterial properties of VCO against S. pyogenes compared with those of LA.

Methods

1. Bacteria and coconut oil

The bacteria tested in this study are presented in Table 1 [13·14·15·16·17·18·19·20·21·22·23]. The VCO, "COCODIA" (Koyosha, Hashima-Gifu, Japan) was kindly provided by Quint Corporation (Sakai-Osaka, Japan), which is a sales company. The fatty acid composition of the VCO is documented in Table 2. The rapeseed oil was purchased from the Nisshin OilliO Group Ltd., (Tokyo, Japan) and lauric acid was purchased from Wako Pure Chemical Industries (Osaka, Japan). The VCO and lauric acid were dissolved in water using a vortex mixer for 2 min or ultrasonication for 3 min with a QSONICA Q55 (Waken B-tech, Minoh-Osaka, Japan).

Table 1. Bacterial strains tested in the present study

Bacteria	Strain	Reference
Staphylococcus aureus	Cowan I	13
	ATCC 6538P	14
Streptococcus agalactiae	A909	15
Streptococcus mutans	MT8148	16
Streptococcus pyogenes	NIH35	17
	SSI-1	18
Streptococcus salivarius	HHT	19
Streptococcus sanguinis	ATCC10558	20
Echerichia coli	ATCC 35218	21
Klebsiella oxytoca	K7	21
Klebsiella pneumoniae	ATCC 4352	22
Pseudomonas aeruginosa	ATCC 27853	23
Serratia marcescence	ATCC 8100	21

Table 2. Fatty acid composition in VCO in this study

Fatty acid	Percent Composition
lauric acid	47%
myristic acid	19%
caprylic acid	9%
palmitic acid	8%
capric acid	6%
oleic acid	6%
stearic acid	3%
caproic acid	1%
linoleic acid	1%

2. Culture of bacteria, and the disk diffusion antibacterial test

The bacteria tested in this study were supplemented with a stock solution that contained 1 volume of 2 × concentrated Todd's Hewitt broth medium (BD Biosciences, Franklin Lakes, NJ, USA) supplemented with 0.2% yeast extract (THY broth medium) and 1 volume of glycerol, and was stored at –20 ° C until use.

The stored bacteria were grown for 14 h at 37 ° C in THY broth medium, after which optical density at 600 nm (OD_{600}) of the culture was adjusted to 0.132

(McFarland standard No. 0.5) by adding THY broth medium. The bacteria (1 μL containing 1–3 × 10^5 CFU) were inoculated and spread onto a Mueller-Hinton (MH) agar medium. Sterilized paper disks (diameter: 8 mm; Advantec, Tokyo, Japan) containing 40 μL of VCO, LA, or rapeseed oil were placed on the bacteria-inoculated agar plates. The plates were then incubated at 37 °C overnight. After the incubation, the size of the bacteria-inhibiting zone surrounding the VCO-containing disks was measured.

3. Antimicrobial test by the inhibition of microbial growth in the broth medium

The stored bacteria were grown for 14 h in THY broth medium, and 100 μL of the culture medium containing the bacteria (1–3 × 10^5 CFU) were inoculated into the MH broth medium containing the indicated concentrations of either VCO or lauric acid. At the time indicated, the bacterial suspensions were serially diluted by 10-fold dilutions, and each diluted bacterial suspension was plated on the MH agar plates. After 24 h of incubation at 37 °C, the number of the bacterial colonies on each plate was enumerated.

4. A scanning electron microscopic (SEM) observation

The method of sample preparation and observations via a scanning electron microscope was performed as described previously [24]. In brief, the stored bacteria were grown for 14 h in THY broth medium, and 4 × 10^6 CFU of bacteria were inoculated into the MH broth medium containing the indicated concentrations of either VCO or LA. Following the incubation, 50 μL of the bacterial samples were poured onto a glass coverslip pre-coated with Matrigel (BD Biosciences), followed by fixation of the samples with 2.5% glutaraldehyde on the glass for 1 h at room temperature. After washing with distilled water, samples were dehydrated with 100% γ-butyl alcohol and freeze-dried. Finally, samples were coated with platinum and examined with a scanning electron microscope (JSM-6390LVZ, JEOL Ltd., Tokyo, Japan).

5. Statistical evaluations

The efficacy of VCO on the growth of S.pyogenes was analyzed by one way ANOVA followed by Dunnett`s post-hoc test. To analyze the data in the other experiments, Student's t-tests were performed. The test was performed using the Stacel2 software (OMS, Tokyo, Japan) [25] and p values < 0.05 were considered to be significant.

Results

1. Comparison of the antimicrobial activity and spectrum of VCO and LA

We examined the antimicrobial spectrum of VCO using a disk diffusion antimicrobial test. A bacteria-inhibiting zone surrounding the paper disk containing VCO was observed on plates inoculated with both S. pyogenes strains, NIH35 and SSI-1 (Table 3). As a control, the inhibiting zone was not formed by rapeseed oil, which possesses no antimicrobial activity. We also observed a bacteria-inhibiting zone around the disk containing 0.17 μmol/40 μL of VCO on plates inoculated with S. pyogenes, S. agalactiae, S. mutans, and S. sanguinis; however, this zone was not present on those containing S. aureus, S. salivalius, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, or Serratia marcescence (Table 3).

LA is a medium-chain fatty acid and comprises nearly 50% w/w of VCO. Since several reports have demonstrated that LA possesses antimicrobial properties [7・8・9・10・12], we sought to determine whether the observed antimicrobial activity and spectrum of VCO was comparable to those of LA. In Table 3, it can be noted that the bacteria-inhibiting zone surrounding the paper disk containing 0.17 μmol/40 μL or 0.085 μmol/40 μL of LA on plates inoculated with S. pyogenes, S. agalactiae, S. mutans, and S. sanguinis was larger than that of the paper disk containing 0.17 μmol/40 μL of VCO. Furthermore, LA demonstrated antimicrobial activity against both the bacterial strains inhibited by VCO, as well as S. aureus and S. salivarius (Table 3). These results indicate that the antimicrobial activity and spectrum of VCO and LA are different.

2. Antimicrobial efficacy on VCO against S. pyogenes

We found that the antimicrobial activity of VCO is limited to the genus Streptococcus. This result raised the question of how VCO mediates such antibacterial effects against the genus Streptococcus. To this end, we performed a detailed analysis of the antimicrobial effect of VCO against S. pyogenes. We prepared broth medium containing various concentrations of VCO by mixing with a vortex for 2 min. Subsequently, the suspensions of S. pyogenes were inoculated onto the media and incubated for 6 h. In Figure 1, in contrast to addition of 0-mM VCO, the addition of more than 4.4 mM VCO was observed to significantly inhibit bacterial growth, and concentrations higher than 110-mM VCO reached a plateau of bacterial

Table 3. Antibacterial activity of VCO assessed via a disk diffusion antibacterial test

Bacteria	Strain	VCO 0.17 μmol (mm)	Rapeseed oil 0.12 μmol (mm)	Lauric acid 0.17 μmol (mm)	Lauric acid 0.085 μmol (mm)
Staphylococcus aureus	Cowan 1	0	0	14	16
	ATCC 6538P	0	0	12	14
Streptococcus agalactiae	A909	34	0	50	48
Streptococcus mutans	MT8148	34	0	80	30
Streptococcus pyogenes	NIH35	40	0	50	54
	SSI-1	24	0	54	40
Streptococcus salivarius	HHT	0	0	82	20
Streptococcus sanguinis	ATCC10556	35	0	90	58
Escherichia coli	ATCC 35218	0	0	0	0
Klebsiella oxytoca	K7	0	0	0	0
Klebsiella pneumoniae	ATCC 4352	0	0	0	0
Pseudomonas aeruginosa	ATCC27853	0	0	0	0
Serratia marcescence	ATCC 8100	0	0	0	0

growth inhibition.

Antimicrobial activity is mediated by either bactericidal or bacteriostatic effect. Therefore, we sought to determine whether the antibacterial activity of VCO against S. pyogenes was due to bactericidal or bacteriostasis. We prepared the broth medium with or without 4.4-mM VCO, and S. pyogenes were inoculated into the media and incubated for 0, 2, 4, 6, 9, or 12 h. As shown in Figure 2, the number of bacteria in the culture medium containing VCO increased from 0 to 2 h after the inoculation; however, the growth range was found to be smaller than that without VCO. Furthermore, from 2 to 9 h after the inoculation, the

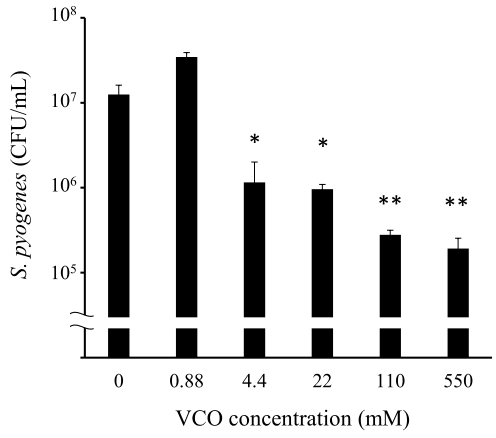

Figure1 The efficacy of VCO on the growth of Streptococcus pyogenes. S. pyogenes (3×10^5 CFU) was inoculated into 4 mL of the MH broth medium mixed with the indicated concentrations of VCO and incubated for 6 h at 37℃. After the incubation period, the bacterial colonies were ennumerated as described in the Materials and Methods section. *; $p < 0.05$ compared with the value of in the absence of VCO (VCO 0 mM). **; $p < 0.01$ compared with the value of 0 mM of VCO.

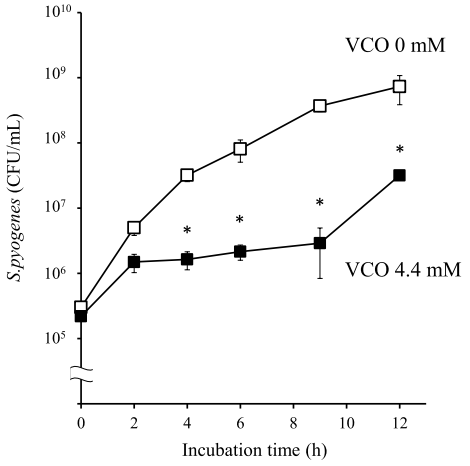

Figure2 Time course change for the suppression of S. pyogenes growth by VCO. S. pyogenes (3×10^5 CFU) was inoculated into 4 mL of the MH broth medium mixed with 4.4 mM or 0 mM of VCO and incubated at 37℃. At the indicated time points, the culture aliquots were plated on agar plates and the number of the grown colonies was ennumerated as described in the Materials and Methods section. *; $p < 0.05$ compared with the value of 0 mM of VCO.

number of the bacteria in the medium containing VCO was maintained. The number of bacteria then increased 12 h after the inoculation.

3. Antimicrobial efficacy of VCO against S. pyogenes via a mixing procedure

It is difficult to dissolve VCO into a water-based solution, and mixing using a vortex does not thoroughly dissolve the VCO. Since a previous study demonstrated that ultra-sonication could mix VCO into a water-based solution, we considered whether the antimicrobial efficacy of VCO could be altered by the mixing procedure. We prepared a VCO solution mixed using either a vortex mixer or an ultrasonicator, and the antimicrobial effect of the two VCO solutions was compared. Figure 3 shows that both solutions demonstrated the same antimicrobial activity; thus, the antimicrobial effect exhibited by VCO is independent of the mixing procedure that was used.

4. Comparative antibacterial effect between VCO and LA

While a concentration of greater than 4.4-mM VCO was capable of exhibiting an antimicrobial effect against S. pyogenes, the same effect was demonstrated by as little as 0.18-mM lauric acid (Figure 4). Furthermore, a concentration higher than 0.88-mM lauric acid, but not VCO, was able to eliminate S. pyogenes completely (Figure 4). The antibacterial mechanism of LA has been shown to be attributed to the ability to lyse bacterial organisms [7・26・27]. We also confirmed that LA could eliminate bacteria within 10 min, and the number of bacteria did not increase for 2 h (Figure 5). On the other hand, the addition of VCO did not decrease the number of bacteria (Figure 5).

These findings raised the question of whether the antibacterial effect of VCO was mediated by sterilization as observed by LA or by another mechanism. To answer this question, we observed the features of the bacteria

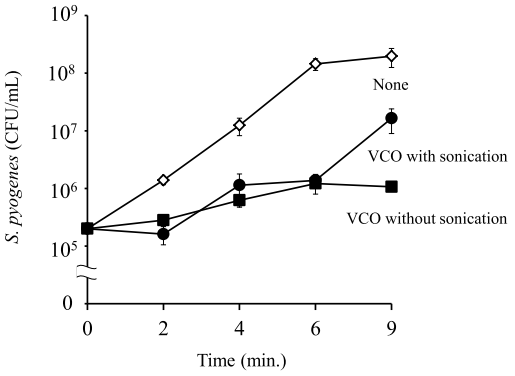

Figure3 Efficacy of the VCO mixing procedure used with the culture medium on the growth suppression of S. pyogenes. S. pyogenes (2×10^5 CFU) was inoculated into 4 mL of the MH broth medium mixed with 4.4-mM VCO using a vortex for 2 min (without sonication) or ultrasonication for 2 min (with sonication), and the solutions were incubated at 37℃. At the indicated time points, the culture aliquots were harvested and the number of the bacterial colonies in the solutions was ennumerated as described in the Materials and Methods section.

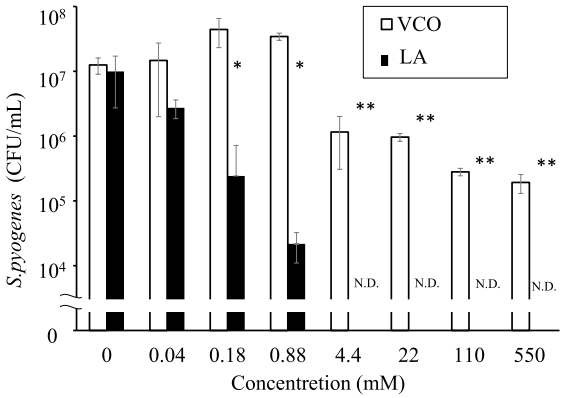

Figure4 Comparative efficacy between LA and VCO on the growth of S. pyogenes. S. pyogenes (3×10^5 CFU) was inoculated into 4 mL of the MH broth medium mixed with the indicated concentrations of VCO or LA, and incubated for 6 h at 37℃. After the incubation, the number of the bacterial colonies was ennumerated as described in the Materials and Methods section. *; $p < 0.05$ compared with the value of LA. **; $p < 0.01$ compared with the value of LA.

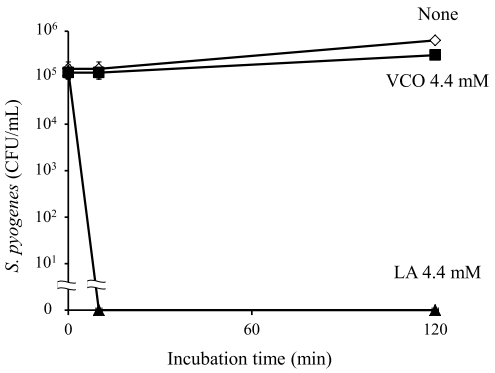

Figure5 Time course changes in the suppression of S. pyogenes growth by VCO or lauric acid. S. pyogenes (10^5 CFU) was inoculated into 4 mL of the MH broth medium mixed with 4.4 mM of VCO or LA, and incubated at 37℃. At indicated time points, the culture aliquots were harvested and the number of bacterial colonies in the solutions was enummerated as described in the Materials and Methods section.

following treatment with LA or VCO. The addition of LA was found to lyse the bacterial organisms, whereas adding VCO did not (Figure 6A). Furthermore, in the expanded photos, the shape of the bacteria following treatment with VCO was smooth, similar to a coating, which differed from the shape of the non-treated bacteria (Figure 6B).

Figure6A

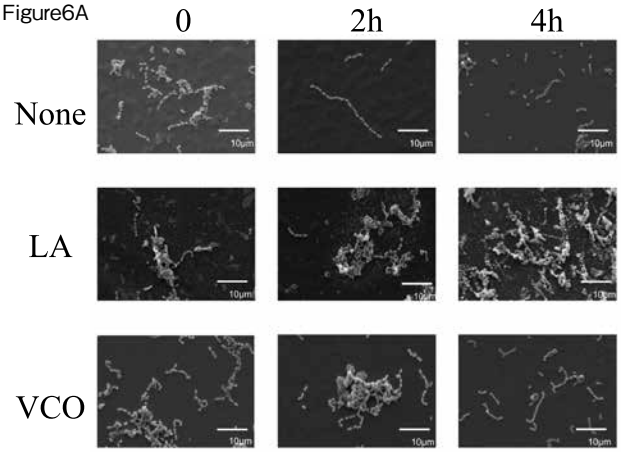

Figure6B

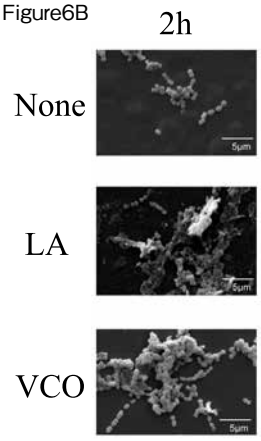

Figure6
Morphological changes of S. pyogenes cells treated with VCO or LA. S. pyogenes was inoculated into the MH broth medium mixed with 4.4-mM VCO or LA and incubated at 37℃. At 0, 2 and 4 hours after the incubation, the solutions were harvested and the moephology of bacteria was observed by a scanning electron microscope. A; Bacterial image at×2,500 magnification. B; Bacterial image at×5,000magnification.

Discussion

VCO consists of 50% w/w LA and seven other medium-chain fatty acids. LA is known to exhibit antimicrobial activity against some strains of bacteria (i.e., Streptococcus, Staphylococcus, and Clostridium) [7]. Therefore, it is believed that VCO should demonstrate antimicrobial activities similar to those of LA. However, some studies have demonstrated that VCO does not display antimicrobial activity against Staphylococcus aureus or Clostridium [11・12]. These findings question whether VCO has antimicrobial properties and if the antimicrobial activity exhibited by VCO is the same as that demonstrated by LA. In the present study, we found that VCO has antimicrobial properties against some strains of bacteria belonging to the genus Streptococcus, but not Staphylococcus aureus or some gram-negative bacteria. These findings suggest that the antimicrobial spectrum of VCO differs from that of LA. We also determined that the antimicrobial effect of VCO is mediated by bacteriostasis, not via bactericidal as induced by LA. Taken together, we suggest that the antimicrobial effects and mechanism of VCO completely differ from those of LA.

The reason that the antimicrobial activity of VCO is different from that of LA remains elusive. VCO contains LA, as well as caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, oleic acid, and linoleic acid. This fact raises the question of whether LA in combination with the other medium-chain fatty acids can alter the quality or magnitude of the antimicrobial properties of lauric acid. Since few reports have investigated this question, future research should focus on these aspects.

In the present study, we demonstrated that the addition of VCO suppressed the growth of S. pyogenes for 10 hours. The growth of bacteria in the broth medium culture is modeled by four distinct phases: 1) lag phase; 2) log phase; 3) stationary phase; and 4) death phase. It is well known that bacteria synthesize RNA, proteins, and enzymes that are required for bacterial growth during the lag phase when the bacteria prepare to start growing [28]. Furthermore, the expression of transporters that are needed for the incorporation of carbohydrates and nitride is enhanced, resulting in the initiation of bacterial growth in the log phase [28]. We predict that following the exposure to VCO, some bacterial species experience a functional impairment during the lag phase, resulting in suppression of growth in the log phase. However, the presence of factors involved in this impairment mediated by VCO remains unknown and should be investigated in future studies.

The genus Streptococcus is divided into six groups: anginosus; bovis; mitis; mutans; pyogenes; and salivarius, according to the difference in the 16S ribosomal RNA [29]. In the present study, we demonstrated that VCO exhibits antimicrobial activity against all tested streptococcal bacteria within the mitis, mutans, and pyogenes groups. Although we found that the antimicrobial activity of VCO against S. pyogenes was mediated by bacteriostasis, we have not determined whether bacteriostasis is also the main mechanism against the other streptococcal bacteria.

The genomic homology of the four bacterial groups is greater than 70%, and there are several proteins and enzymes that all six streptococcal groups have in common. Therefore, we speculate that the antimicrobial activity of VCO against the other streptococcal bacteria may also be mediated by bacteriostasis.

While the antimicrobial activity of VCO was demonstrated against streptococcal bacteria, this effect was not observed against S. aureus or Gram-negative bacteria. This discrepancy in the antimicrobial effect of VCO against different bacterial species may be due to the method of inducing bacteriostasis. The major bacterial genus Streptococcus, particularly the mitis, mutans, and pyogenes groups, exists in the oral and pharynx area and is responsible for several illnesses (i.e., dental caries, several oral mucosal diseases, bacterial endocarditis, and pharyngitis) [18・30・31・32・33・34]. In contrast, many other types of bacteria with rare pathogenicity including S. salivarius, that exist in the oral and pharyngeal area form the natural bacterial flora in these regions 35. If antibiotics are administrated to eliminate streptococcal bacteria in the oral and pharyngeal area, several other strains of bacteria in the area are also eliminated, resulting in the enhanced susceptibility to microbial substitution diseases. Thus, VCO might function to control streptococcal bacteria without microbial substitution diseases and might help protect against several infectious diseases of the oral and pharyngeal area.

Coconut oil, including VCO, decreases cardiovascular risk factors and is protective against heart disease [2]. It has also been reported that VCO could function as an efficient nutraceutical in preventing the development of diet-induced insulin resistance and associated complications via its antioxidant properties [6]. In this study, we demonstrated that VCO displays novel bacteriostatic activity against streptococcal bacteria. In the future, the bacteriostatic mechanism of VCO and should be determined, and the discrepancy of the antimicrobial activity between VCO and LA should be clarified.

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オーガニックバージンココナッツオイルとラウリン酸における細菌に対する抗菌作用の範囲とその作用機序の比較

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要　　旨

オーガニックヴァージンココナッツオイル（VCO）は、およそ50%ものラウリン酸を含有している。ラウリン酸（LA）は、いくつかの細菌種に対して抗菌作用を呈することから、VCOも抗菌作用を有すると考えられている。しかし、VCOがラウリン酸と同等の抗菌効果を示すか否かは明らかではない。今回我々は、VCOがLAと同等に数種類のグラム陽性細菌に対する抗菌作用を示すかディスク法を用いて検討した。LAは、黄色ブドウ球菌、A群化膿性レンサ球菌、B群レンサ球菌、ストレプトコッカス・ミュータンス、ストレプトコッカス・サングイニスに対する抗菌効果を示したが、VCOは、黄色ブドウ球菌に対する抗菌効果を有さず、残りの菌種に対する抗菌効果もLAと比べて効果は弱小であった。次に我々はA群化膿性レンサ球菌を用いて液体培地での細菌培養系におけるVCOおよびLAに対する増殖抑制効果について比較検討を行った。その結果、VCOでは4.4 mM以上の濃度でないと抗菌効果を発揮しないのに対し、LAでは0.18 mM以上の濃度で同等の抗菌効果を発揮した。また、LAでは、わずか10分で細菌を死滅させる一方、VCOは死滅させないことを見出した。また、走査型電子顕微鏡による観察でLAによる抗菌効果は菌体破壊による殺菌効果によるものに対し、VCOは、菌体破壊のない静菌効果によるものであることを見出した。以上より、VCOが一部のレンサ球菌属に対して抗菌効果を示し、黄色ブドウ球菌には抗菌効果を示さないこと、VCOによる抗菌効果が静菌効果によることを明らかにし、VCOの抗菌効果がLAと異なることを示唆した。